Aflatoxin Detection and Determination in Corn

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ABSTRACT

There are three approaches to aflatoxin detection in corn: (1) a presumptive test that can be done in the field to determine whether a corn lot should be analyzed for aflatoxin or not; (2) rapid screening methods to establish the presence or absence of toxin; and (3) quantitative methods to determine toxin levels. The presumptive test for aflatoxin in corn is the black light test or the bright greenish-yellow fluorescent test based on the fluorescence under ultraviolet light (365 nm) associated with *Aspergillus flavus* or *A. parasiticus*. Rapid screening tests have included minicolumn methods that can be done in a laboratory with minimal facilities and thin-layer chromatography (TLC). Determination of aflatoxin levels involves extraction, purification of the extract, and measurement of the toxin by TLC using visual comparisons with a standard or densitometry or high-pressure liquid chromatography. A fluorometric-iodine method has been used both for screening purposes and for quantitation of aflatoxin in corn.

INTRODUCTION

Because of aflatoxin formation in the field, the presence of the mycotoxin in some corn is unavoidable at this time. Therefore, it is important to have reliable procedures available for determining its occurrence and contamination levels. When it became obvious that aflatoxin could be a problem in corn, there had already been a number of analytical methods developed for peanuts and cottonseed. Some of these methods were applicable for the detection and determination of aflatoxin in corn; some were not. The methods vary in purpose and complexity. Aflatoxin detection methods can be divided into three categories: (1) rapid presumptive tests to identify corn lots that may contain the toxin; (2) rapid screening procedures to determine the presence or absence of toxin, and (3) quantitative methods to determine aflatoxin levels.

BRIGHT GREENISH-YELLOW FLUORESCENT PRESUMPTIVE TEST FOR AFLATOXIN IN CORN

The basis of the presumptive test for aflatoxin in corn known as the bright greenish-yellow fluorescent (BGYF) or the "black light" test is the characteristic fluorescence under long-wave ultraviolet light (365 nm) associated with the presence of *Aspergillus flavus* or *A. parasiticus*, aflatoxin-producing fungi, or possibly the mycotoxin itself. The BGYF test indicates the growth of the fungi that may have resulted in the production of aflatoxin. BGYF was first observed in naturally contaminated corn samples collected in 1969-1970 from the South *(545)*. Inoculation of living corn kernels with *A. parasiticus* NRRL 2999 and incubation resulted in the formation of BGYF *(394)*. Marsh postulated that BGYF resulted from the action of heat labile peroxidase in the living plant (cotton fibers, corn, etc.) on kojic acid produced by *A. flavus* species possibly concurrently with aflatoxin. When Marsh et al. treated aqueous solutions of kojic acid with peroxidase, hydrogen peroxide, or potassium permanganate, BGYF formed. However, *A. parasiticus* NRRL 2999 produced BGYF on glucose-mineral salts medium with long incubation even without peroxidase. Inoculation of autoclaved (nonliving) corn kernels simultaneously with *A. flavus* NRRL 6412 and certain fungal isolates from corn *(Alternaria alternata, Cladosporium cladosporoides, Curvularia lunata, Fusarium moniliforme, Penicillium variabile*, and unidentified yeast) followed by incubation resulted in the formation of BGYF *(648)*.

Analysis of BGYF kernels from ten corn lots revealed aflatoxin B_1 levels of 284-101,000 μg/kg *(545)*. In the same study, kernels with white, blue, or orange fluorescence under UV light (365 nm) contained no aflatoxin. Good correlation was observed between the presence of BGYF, *A. flavus*, and aflatoxin in a lot of aflatoxin-contaminated white corn *(201)*. Fluorescing kernels separated from 50 g of the white corn contained 7,500 μg/kg aflatoxin.

Inoculation of harvested corn with *A. parasiticus* or *A. flavus* led to production of BGYF and aflatoxin in the laboratory *(83, 492)*. Others observed the formation of BGYF and sometimes aflatoxin after inoculation of corn ears in the field with *A. flavus* or *A. parasiticus* strains *(23, 364, 367, 491)*.

Basically, the BGYF test is the inspection of a 10-lb corn sample under black or long-wave ultraviolet light (365 nm) in a darkened chamber or room. A high-intensity light is recommended, but lower intensity lights may be used in complete darkness. Goggles that screen out UV light lessen eye strain and prevent possible eye damage from continued exposure. The test is easily adapted for field use. False positive results may be reduced by use of the color standards Tinopal BHF (Ciba-Geigy Corporation, Greensboro, NC 27409) or a Blak-Ray green fluorescing crayon (Ultra-Violet Products, Inc., 5100 Walnut Grove Avenue, San Gabriel, CA 91778). BGYF has a bright glow, sometimes called a firefly glow, that differentiates it from other fluorescent materials in corn. Examples of possible interferences with the BGYF test are a light yellow fluorescence of the tips of corn kernels, cob tips, and glumes; the dull green-yellow fluorescence of soybeans without seed coats; and the bright green-yellow fluorescence of jimson weed at the hilum end or without seed coats *(604)*. Authentic BGYF from corn kernels is water soluble, whereas BGYF from glumes and cob tips is not. The fluorescent material of jimson weed is water soluble, but the seed can usually be identified. When a BGYF positive corn sample is encountered, it should be further analyzed for aflatoxin.

It has been recommended that corn be cracked or coarsely ground before critical BGYF inspection because the fluorescence can occur under the seed coat *(546)*. Sometimes it can be detected as a dull gold color under the seed coat, usually in the germ area, and becomes fully visible when cracked. In the study leading to this recommendation, 31 percent of the 86 kernels with fluorescence under the seed coat had less than 100 μg/kg, but three kernels had 122,000, 187,000, and 207,000 μg/kg aflatoxin B_1. BGYF can be fully hidden within the kernel and be detected only when the kernel is broken. Most of the corn samples that have been encountered with aflatoxin levels equal to or more than 20 μg/kg have had at least one or two kernels with visible BGYF per kg sample. Corn can be cracked or coarsely ground in a Straub disc mill, coffee grinder, or Tag-Heppenstahl moisture meter equipped with a large soybean shim before BGYF inspection.

A black light viewing apparatus (CPC International, Argo, Ill.) has been described by Barabolak et al. *(50)* in which corn is

[1]The mention of firm names or trade products does not imply that they are endorsed by the U.S. Department of Agriculture over other firms or similar products not mentioned.

discharged in a monolayer onto a vibrating feeder tray moving under ultraviolet light (365 nm). The feeder tray and light were enclosed in a cabinet with a viewing port. A comparison was made of the number of BGYF particles and kernels observed in 10-lb corn samples in the black light viewer and the number of BGYF particles counted in the stream from the Straub disc mill as the same samples were coarsely ground *(551)*. It was concluded that the BGYF test can be carried out equally well by using the black light viewer on whole kernel corn or by inspecting a stream of coarsely ground corn from a mill under ultraviolet light (365 nm). In laboratories where efficient exhaust fume hoods are available, examination of streams of coarsely ground corn is convenient, and the process facilitates the preparation of finely ground subsamples for aflatoxin analysis. However, in an elevator in the field, use of the disc mill is difficult as well as hazardous to unskilled workers. The grinding process creates dust under circumstances where dust is highly undesirable. If the corn dust contains aflatoxin, it presents a potential hazard to workers inhaling it.

Because the BGYF test is so easily done, attempts have been made to establish aflatoxin levels by numbers of BGYF particles present or by the weight of BGYF particles in whole kernel samples. BGYF particles and kernels were counted in 10-lb samples of whole kernel white corn harvested in southeastern Missouri, and counts were compared with aflatoxin levels in the same samples, Table 1. Although a relationship existed between numbers of BGYF particles and kernels and aflatoxin levels, the correlation was not high enough to encourage use of the numbers as an indication of aflatoxin content *(548)*. In fact, 35 percent of the samples that had more than four BGYF particles and kernels per kg had less than 20 μg/kg aflatoxin. Barabolak et al. *(50)* also support the conclusion that BGYF count is not a quantitative measure of aflatoxin levels in yellow corn samples. They found that only one out of three lots having more than four BGYF particles per kg had more than 20 μg/kg aflatoxin.

The number of BGYF particles was compared with aflatoxin levels in 1973 South Carolina yellow corn samples *(366, 551)*. Although there was a relationship between BGYF counts and aflatoxin levels, there was no indication from the results in Tables 2 and 3 that aflatoxin levels could be determined with certainty by the number of BGYF particles in a given sample. However, considering the results on 1971 white corn from Missouri, 1973 yellow corn from South Carolina, and 1978 corn (of 248 samples, only six were white corn), a count of one BGYF particle per kg obtained on a given corn sample indicates that the sample should be tested for aflatoxin by chemical means.

There have been two studies on the prediction of aflatoxin levels in corn by the weight of corn particles exhibiting BGYF. In the first, 1,283 truckloads of 1971 white corn were sampled as they were delivered from 61 farms in six southeastern Missouri counties *(336)*. Three models were considered for prediction equations: (I)Y = RX, (II)Y = CX^D, and (III)Y = A + BX. The aflatoxin level was Y in μg/kg; the weight percent of BGYF was X; and R, C, D, A, and B were constants whose values were determined by the least square means. The relation between BGYF weight and aflatoxin level was highly dependent on the farm, and for each farm the association was positive. A summary of results for 33 farms where five or more truckloads were delivered is shown in Table 4. The mean levels by farm, and the simple linear correlation of BGYF and total aflatoxin (Equation III), are shown in columns 3-5. For 12 of 33 farms, the correlation was significant. The value for A varied from -49.08 to 23.30, and for B, from -249.53 to 1,121.88. Results of Equations I and II were similar. Values for R ranged from 0 to 1,915 with an overall mean of 287. The variability suggests that an estimate of aflatoxin levels based on weight of BGYF particles is too imprecise for practical use.

In a second study, the weight percent of BGYF (wt. pct. BGYF) particles and aflatoxin levels in yellow corn lots taken in 1977 and 1978 in North Carolina were determined within a week after harvest to study the relationship between the two *(165)*. In one test, 113 of 250 samples collected in 1977 contained BGYF kernels. The BGYF and non-BGYF (NBGYF) portions from each of these samples were weighed and analyzed for aflatoxin. Average data for the test are given in Table 5. The average aflatoxin concentration in NBGYF was 49 μg/kg; in BGYF kernels was 8,665 μg/kg; and in total samples was 79 μg/kg. If one assumes that all of the aflatoxin is confined to the BGYF fractions, visible and hidden in the kernels, and average aflatoxin level in visible and hidden BGYF is the same, then Equation I applies.

(I) μg/kg in sample = pct. BGYF in sample (8,665 μg/kg)/100.

As the average aflatoxin concentration in the total samples was 79 μg/kg, solution of Equation I indicates that the equivalent average

TABLE 1. RELATION OF AFLATOXIN LEVEL TO NUMBER OF BGY FLUORESCING PARTICLES[a] AND KERNELS IN UNGROUND SAMPLES

Aflatoxin Level ppb[b]	Number of BGY fluorescing particles and kernels			
	0	1-3	4-20	>20
0	88[c]	60	41	6
<10	7	14	20	14
10-19	3	10	17	15
20-29	1	6	11	7
30-100	1	9	11	34
>100	0	0.3	0	24

[a]Bright greenish-yellow fluorescence under ultraviolet light (365 nm).
[b]As determined at NRRC by AOAC Official First Action Method.
[c]Percentage of total sample.

TABLE 2. COMPARISONS OF AFLATOXIN LEVELS AND BGY[a] PARTICLES AND KERNELS PER KILOGRAM IN UNGROUND 1973 SOUTH CAROLINA CORN SAMPLES[b]

Total aflatoxin level (ng/kg)	Number of BGY particles and kernels per kilogram									
	None		<1		1		2		>2	
	N[c]	Pct.	N	Pct.	N	Pct.	N	Pct.	N	Pct.
ND[d]	72	88	33	65	37	47	3	10	0	0
<20	8	10	13	25	24	30	8	25	2	4
20-49	2[e]	2	4	8	16	20	11	34	15	28
50-99			1[f]	2	2	3	8	25	15	28
100-500							2	6	18	33
>500									4	7
Total	82		51		79		32		54	

[a]Bright greenish-yellow fluorescence.
[b]Of the 82 samples that were BGY-negative when unground, 21 were BGY-positive when coarsely ground.
[c]Number of samples.
[d]Not detected.
[e]These samples had 21 and 27 ng/g total aflatoxin.
[f]This sample had 51 ng/g total aflatoxin.

TABLE 3. COMPARISONS OF AFLATOXIN LEVELS AND BGYF[a] PARTICLES IN COARSELY GROUND 1978 CORN

Total aflatoxin level (ng/kg)	BGY particles per kilogram									
	1		2		3		4		>4	
	N[b]	Pct.	N	Pct.	N	Pct.	N	Pct.	N	Pct.
ND[c]	41	63	17	57	9	41	4	36	9	8
<20	19	29	5	17	7	32	3	27	16	15
20-49	1	2	6	20	2	9			19	18
50-99	4	6	1	3	3	14	2	18	12	11
100-500			1	3	1	4	1	9	35	33
>500							1	9	16	15
Total	65		30		22		11		107	

[a]Bright greenish-yellow fluorescence.
[b]Number of samples.
[c]Not detected.

TABLE 4. SUMMARY OF BGYF[a]—AFLATOXIN DATA BY FARM

Farm	Number of samples	Mean Pct. BGYF X 100	Mean Total aflatoxin	Correlation[b] (r)	Y[c] = A + BX[d] A	B
1	27	72.31	28.74	0.83**	0.90+	38.502
2	7	4.85	5.14	0.91**	-3.28+	173.734
3	30(5)[e]	19.52	63.70(12.4)[f]	0.49**	34.11+	151.580
4	9	13.27	46.33	0.57		169.21[g]
5	7	3.48	35.14	0.88**	-1.92+	1,065.583
6	36(3)	8.14	9.39(3.3)	0.56**	3.11+	77.08
7	12(2)	44.42	257.00(8.0)	-0.32		-249.53
8	15	1.47	2.27	-0.48		-114.82
9	10	4.59	13.80	0.51		134.23
10	16	3.53	11.63	0.72**	4.11+	212.88
11	5	2.38	3.48	-0.57		-89.81
12	7(3)	9.79	60.71(16.7)	0.95**	-49.08+	1,121.88
13	10	3.80	8.90	0.12		37.46
14	15	0.46	12.40	0.05		50.34
15	5	0	20.00	0		0
16	9	4.04	8.22	0.33		70.90
17	6	0.16	8.50	0.42		435.74
18	5(1)	31.46	61.40(4)	0.07		13.69
19	10	20.18	151.80	0.85**	19.38+	656.34
20	8(5)	17.64	60.38(27.6)	0.97**	10.86+	280.68
21	10(1)	19.35	33.10(5)	0.60		123.55
22	9	0	46.89	0		0
23	30(1)	20.18	14.80(1)	0.81**	1.36+	66.63
24	11	6.96	2.09	0.42		16.12
25	23	0.96	12.74	0.17		184.65
26	6	8.85	32.33	0.76		169.55
27	10	12.56	108.90	0.40		692.51
28	12	4.88	14.58	0.66*	7.22+	150.89
29	7	17.83	24.29	0.72		208.92
30	6	2.47	0	0		0
31	18(3)	7.45	68.11(12.3)	0.86**	22.30+	614.77
32	12	3.65	13.83	0.44		175.16
33	6	0.54	2.67	0.25		70.47

[a]BGYF = bright greenish-yellow fluorescence.
[b]**Significant at 0.01 level.
*Significant at 0.05 level.
[c]Y = Aflatoxin $B_1 + B_2 + G_1 + G_2$.
[d]X = Wt. pct. BGYF particles.
[e]Number of positive G_1 samples.
[f]Mean G_1 level.
[g]Slope of equation (nonsignificant correlation).

TABLE 5. DETERMINATIONS FOR THE 113 SAMPLES OF CORN USED IN TEST 1

Average wt. of samples	3,924 g
Average aflatoxin concentration in NBGYF[a] kernels	46 ppb
Average aflatoxin concentration in BGYF kernels	8,665 ppb
Average aflatoxin concentration in total sample	79 ppb
Average wt. pct. BGYF kernels in samples	0.38 pct.
Average pct. of total aflatoxin in BGYF kernels	41.9 pct.
Average pct. of total aflatoxin in NBGYF kernels	58.1 pct.
Average no. of fluorescent particles/cm² of surface	1.41

[a]NBGYF = non bright greenish-yellow fluorescence.

wt. pct. BGYF in the samples was 0.91 percent rather than 0.38 percent determined by hand-sorting of whole kernels. Equation II derives a relationship between the total amount of BGYF kernels in the sample and the amount of BGYF removed by hand-sorting.

(II) pct. BGYF in samples = $\frac{0.91}{0.38}$ (pct. BGYF determined by hand-sorting) = 2.39

Substitution of 2.39 into Equation I leads to the following relationship between aflatoxin concentration of the samples and the average wt. pct. BGYF kernels determined by hand-sorting:

(III) µg/kg in samples = 207 X pct. BGYF determined by hand-sorting.

After the wt. pct. BGYF kernels was determined in a second test (*165*), the BGYF portion was added back to each of 2,387 samples of corn collected in 1977 and 1978. Aflatoxin concentrations were determined in the samples. If aflatoxin in corn samples is confined to BGYF portions, a plot of aflatoxin concentration vs wt. pct. BGYF is linear with no intercept. Equation IV is a linear regression equation with no intercept for 2,304 data points.

(IV) µg/kg in samples = 197 X pct. BGYF determined by hand-sorting.

The remaining 83 data points from 2,387 samples were removed from the data set as outliers because their observed values deviated from predicted values more than ±2.5 standard deviations. Equations III and IV were in agreement.

Comparisons of acceptance levels obtained by the BGYF screening method and the chemical assay method are shown for 2,304 corn lots in Table 6. A comparison of results from acceptance levels of ≤ 20 µg/kg, ≤ 50 µg/kg, or ≤ 100 µg/kg with acceptance levels of ≤ 0.10%, ≤ 0.25%, or ≤ 0.50%, respectively, for the BGYF method indicates that both methods rejected approximately the same percentage of lots tested. However, the difference in average aflatoxin levels for the accepted lots shows the same lots were not rejected by both methods. The authors concluded that more research was required to compare efficacy of the two methods under a variety of conditions.

TABLE 6. COMPARISON OF EFFICACY OF THE BGYF SCREENING METHOD AND THE CHEMICAL ASSAY METHOD TO DETECT AFLATOXIN CONTAMINATION IN 2,304 LOTS OF CORN WITH AN AVERAGE AFLATOXIN CONCENTRATION OF 66 PPB

Chemical assay method			
Aflatoxin concentration in sample when lot accepted (ppb)	<20	<50	<100
Pct. of all lots tested that were accepted	60	73	82
Average aflatoxin concentration in accepted lots (ppb)	4	10	18
Pct. of all lots tested that were rejected	40	27	18
Average aflatoxin concentration in rejected lots (ppb)	157	218	291
BGYF screening method			
Wt. pct. BGYF kernels in sample when lot accepted	<0.1	<0.25	<0.5
Pct. of all lots tested that were accepted	59	72	81
Average aflatoxin concentration in accepted lots (ppb)	10	16	22
Pct. of all lots tested that were rejected	41	28	19
Average aflatoxin concentration in rejected lots (ppb)	148	195	260

RAPID SCREENING METHODS FOR AFLATOXIN IN CORN

A number of rapid screening methods for aflatoxin in corn have been reported involving the use of minicolumns, thin-layer chromatography (TLC), and a fluorometric-iodine method to detect the toxin. Minicolumn screening methods are most widely used, and those reported for aflatoxin in corn are summarized in Table 7. They all include the following steps: extraction, purification of extracts, concentration, and development on a minicolumn. The first minicolumn screening procedure for aflatoxin in corn was reported by Pons et al. (*479*). The purification was by precipitation of impurities with lead acetate from extracts. Concentration of aflatoxin was achieved by transfer into a small volume of benzene. The detection limit of the Pons method was 10 µg/kg.

Velasco (*618*) reported a more complicated method that he later applied to corn. Although it was more time consuming, it was more sensitive with a detection limit of 5 µg/kg. To determine the extent of aflatoxin in commercial lots of marketed corn, slight modifications were made in the Velasco method by the Grain Division, Agricultural Marketing Service, USDA (*297*). During 1972 and 1973, corn samples (10,803) being officially graded were examined for BGYF. BGYF-positive samples were assayed by the modified Velasco minicolumn procedure and 325 were found to contain

TABLE 7. SCREENING METHODS FOR AFLATOXIN IN CORN USING MINICOLUMNS

Extraction solvent	Purification agent	Concentration	Minicolumn components top to bottom	Development	Detection limit (μg/kg)	Reference
Acetonitrile-water (80:20, v/v)	Lead acetate	Liquid-liquid transfer	Silica gel, acidic alumina	Ascending	10	*479*
Acetone-water (85:15, v/v)	Ferric gel	Evaporation	Neutral alumina, silica gel, Florisil	Descending	5	*618, 558*
Acetone-water (85:15, v/v)	Ammonium sulfate	Liquid-liquid transfer	Silica gel, acidic alumina	Ascending	10	*536, 558*
Acetone-water (85:15, v/v)	Ammonium sulfate	Evaporation	Neutral alumina, silica gel, Florisil	Descending	2	*51*
Methanol-water (80:20, v/v)	Zinc acetate	Liquid-liquid transfer	Neutral alumina, Florisil	Descending	5-10	*286*
Methanol-water (80:20, v/v)	Zinc acetate	Liquid-liquid transfer	Neutral alumina, silica gel, Florisil	Descending	5	*535*

aflatoxin. Romer *(508)* developed a minicolumn method to detect aflatoxin in 24 agricultural commodities that was a modification of the Velasco method, which is approved by the American Association of Cereal Chemists *(18)* and the Association of Official Analytical Chemists *(40)*.

Shannon et al. *(536)* substituted acetone-water (85:15, v/v) for the acetonitrile-water (80:20, v/v) and ammonium sulfate for lead acetate in the Pons method to avoid the use of more toxic chemicals. The method was used to monitor 1,283 truckloads of 1971 white corn under Commodity Credit Corporation loans as they were delivered at an elevator in southeastern Missouri *(556)*. Samples to be tested for aflatoxin were selected by the BGYF test. Results of the rapid field method were compared with those from quantitative determinations on the same truckloads and found to be effective in identifying aflatoxin-containing corn.

A collaborative study of the Pons, Velasco, and Shannon minicolumn screening methods was carried out on naturally contaminated and spiked white and yellow corn *(558)*. Eleven laboratories from state and federal agencies and industry participated in the study leading to the approval of the Velasco and Shannon minicolumn methods for aflatoxin in corn in Official First Action by the AACC *(18)* and the AOAC *(40)*. The Velasco and Shannon methods have since been dropped.

The extraction and concentration of Pons et al. *(479)*, the purification of Shannon et al. *(536)*, and the minicolumn of Velasco *(618)* were combined in a method published by Barabolak et al. *(51)*. The method was applicable to corn, corn gluten, gluten feed, and steepwater. The detection limit for corn was 1-2 μg/kg; and for the derived corn products, 5-10 μg/kg. In 1974, the Food and Drug Administration agreed that 1971 white corn still stored in Missouri could be sold for animal feed, if it contained less than 20 μg/kg aflatoxin by this method *(555)*. The method was changed slightly to decrease the sensitivity for the "go—no go" situation. Sixty corn lots (2,200-2,500 bushels) were sampled and tested by the Barabolak minicolumn method as the storage bin was unloaded at the elevator. Twenty lots contained less than 20 μg/kg aflatoxin by the minicolumn method and were sold. Results obtained on the same lots by the CB method indicated that all of the lots sold contained less than 20 μg/kg aflatoxin. Of the 40 corn lots that were rejected by the minicolumn method, nine contained 13-19 μg/kg mycotoxins by the CB method.

In 1977, an outbreak of aflatoxin contamination in Southeastern corn precipitated widespread use of minicolumn screening methods to detect the mycotoxin. Methods used were the ones reported by Barabolak et al. *(51)* and Holaday and Lansden *(286)* and a combination of the two—the Holaday-Velasco method—that used the cleanup procedure of Holaday and Lansden and the Velasco column as reported by Barabolak. The three methods were evaluated in a collaborative study on spiked and naturally contaminated yellow corn *(535)*. Twenty laboratories participated from ten states, the federal government, and industry. The results are shown in Table 8. The limit of detection was 5 μg/kg for more experienced analysts. As a result, the Holaday-Velasco method was approved in Official First Action by the AACC *(21)* and AOAC *(40)*. The Shannon and Velasco minicolumn methods were dropped from the AACC and AOAC books of methods. Most collaborators commented on the difficulty of interpreting the Holaday minicolumn and the time required to carry out the clean up in the Barabolak method.

A combination fluorometer and colorimeter was adapted to measure the fluorescence intensity of aflatoxins on the Florisil layer in the minicolumn *(619)*. It was stated that the detection of 1 ng aflatoxin B_1 was well within the limits of the filter fluorometer with a photomultiplier detector. The relationship was linear from 10 ng to 100 ng standard aflatoxin B_1. The minicolumn method using a filter fluorometer was compared with the CB method for determining aflatoxin in corn *(549)*. Corn samples *(141)* were analyzed by both methods. The minicolumn fluorometer method identified many more samples as aflatoxin-positive than did the CB method. Some corn samples contain a blue-fluorescing substance that migrates between aflatoxin B_2 and G_1 on thin-layer chromatography (TLC) plates and interferes with the minicolumn chromatography of aflatoxins. Measurements by the filter fluorometer of aflatoxin adsorbed on the Florisil layer of minicolumns were used to evaluate the use of water slurries of corn meal for aflatoxin analysis *(620)*.

In laboratories that have the capability of performing TLC, one of the most effective and efficient screening methods is that reported by Dantzman and Stoloff *(149)*. The residual oil from a water-chloroform extraction of corn is spotted on a TLC plate. The plate is first developed with anhydrous ether to move lipid impurities to the solvent front and then dried. The plate is redeveloped in the same direction with chloroform-acetone (90:10, v/v) for aflatoxin. Seventeen extracts can be screened with one standard on a TLC plate (20 X 20 cm). A plate can be used twice if it is developed only 10 cm. When a ground corn sample is extracted, two 50-ml aliquots of chloroform can be collected, one of which can be saved for quantitation if aflatoxin appears to be present by the rapid TLC method. The method is approved by the AOAC *(40)* and the AACC *(17)*. The separation and identification of aflatoxin from interfering lipids in corn oil extracts can be achieved in one rapid TLC development by selecting the proper combination of silica gel and grade of ether (not anhydrous) *(601)*.

A rapid screening procedure for aflatoxin in corn, known as the fluorometric-iodine rapid screen (FL-IRS) method, has been reported *(152)*. Ground corn is extracted with methanol-water (80:20, v/v) and treated with an iodine solution. Fluorescence of the

TABLE 8. COLLABORATIVE RESULTS OF SCREENING PROCEDURES TO DETECT AFLATOXIN IN YELLOW CORN (NG/G)

	CPC method									Holaday method									CPC and Holaday method								
	Blank	Spiked				Naturally contaminated				Blank	Spiked				Naturally contaminated				Blank	Spiked				Naturally contaminated			
Sample	24	55	07	54	79	11	32	31	88	33	18	34	44	68	78	06	67	92	76	73	13	37	93	50	04	39	23
Concn.	(0)	(2.5)	(5.0)	(10.0)	(20.0)	(10.0)	(16.6)	(16.6)	(15.0)	(0)	(2.5)	(5.0)	(10.0)	(20.0)	(10.0)	(16.6)	(16.6)	(15.0)	(0)	(2.5)	(5.0)	(10.0)	(20.0)	(10.0)	(16.6)	(16.6)	(15.0)
Collaborator																											
1	[a]	•	•	•	•	•	•	•	•	ND	•	•	•	•	•	•	•	•	ND	•	•	•	•	•	•	•	•
2	ND[b]	ND	ND	ND	ND	ND	•	•	•	ND	ND	ND	•	•	•	•	•	•	ND	•	ND	ND	•	•	•	•	•
3	ND	ND	•	•	•	•	•	•	•	ND	ND	•	•	•	•	•	•	•	ND	ND	ND	•	•	•	•	•	•
4	ND	ND	•	•	•	•	•	•	•	ND	ND	ND	•	•	•	•	•	•	ND	ND	ND	•	•	•	•	•	•
6	ND	ND	ND	•	•	•	•	•	•	ND	ND	ND	•	•	•	•	•	•	ND	ND	ND	•	•	ND	•	•	•
7	ND	ND	•	•	•	•	•	ND	•	ND	ND	ND	•	•	•	•	•	•	•	ND	ND	•	•	•	•	•	•
8	•	•	•	•	•	•	•	•	•	•	ND	•	•	ND	•	•	•	•	•	ND	•	•	ND	•	•	•	•
9	ND	•	•	•	•	•	•	•	•	ND	•	•	•	•	•	•	•	•	ND	•	•	•	•	•	•	•	•
10	ND	•	•	•	•	•	•	•	•	ND	ND	•	•	•	•	•	•	•									
11	ND	•	•	•	•	•	•	•	•	ND	ND	ND	•	•	•	•	•	•	ND	ND	ND	•	•	•	•	•	•
15	ND	•	•	•	•	•	•	•	•	ND	ND	ND	•	•	•	•	•	•	ND	ND	•	•	•	•	•	•	•
16	•	ND	ND	•	•	•	•	•	•	ND	ND	ND	•	•	•	•	•	•	ND	ND	•	•	•	•	•	•	•
17	ND	•	•	•	•	•	•	•	•	ND	ND	•	•	•	•	•	ND	•	ND	•	•	•	•	•	•	•	•
18	ND	•	•	•	•	•	•	•	•	•	ND	•	•	•	•	•	•	•	ND	ND	•	•	•	•	•	•	•
19	ND	•	•	•	•	•	•	•	•	ND	ND	ND	•	•	•	•	•	•	ND	ND	ND	•	•	•	•	•	•
20	ND	ND	ND	•	•	•	•	•	•	ND	ND	•	•	•	•	•	•	•	ND	ND	•	•	•	•	•	•	•
21	ND	•	•	•	•	•	•	•	•	ND	ND	ND	•	•	•	•	•	•	ND	ND	•	•	•	•	•	•	•
22	ND	•	•	•	•	•	•	•	•	ND	ND	ND	•	•	•	•	•	•	ND	ND	•	•	•	•	•	•	•
23	ND	ND	•	•	•	ND	•	•	•	ND	ND	ND	•	ND	•	•	•	•	ND	•	•	•	•	•	•	•	•

[a] • - +

[b] ND - not detected.

treated solution was compared with that of controls prepared by treating extracts of aflatoxin-free corn with the iodine solution. All samples that fluoresced greater than the controls were labeled aflatoxin positive, whereas those that fluoresced less than the controls were labeled aflatoxin negative. Fluorescence was measured with a Coleman Model 12-C Electronic Photofluorometer (Coleman Instruments Division of Perkin-Elmer, Oakbrook, Ill.) and a filter combination. The PC-6 primary filter (Corning Glass CS7-51) was purchased from Coleman Instruments Division and passed the 365-triplet of the mercury lamp. The secondary filter was made by Spectro-Film (Winchester, MA) and was a 32-mm square X 6.5-mm thick interference filter with a 10-nm band width that peaked at 445 nm. This filter gave 35-50 percent transmission and was made of nonfluorescing glass. Of the 170 samples of corn screened by the FL-IRS method, 119 samples were correctly identified as aflatoxin-negative and 32 were correctly identified as aflatoxin-positive. Only 14 samples screened by the FL-IRS method were false positives and five were false negatives. The quantitative determination of aflatoxin in corn by the fluorometric-iodine method will be discussed later.

A screening test, developed by Seitz and Mohr (*528*), involves extraction of whole or ground corn with methanol in a blender, addition of ammonium sulfate to the extract, distribution or extraction with hexane to remove lipid impurities, and extraction of the defatted aqueous methanol ammonium sulfate solution with methylene chloride. The residue from the methylene chloride solution is used for TLC. The TLC plate is developed only 3 cm (taking 3 minutes), which is enough to separate aflatoxins from interfering substances for identification. The detection limit is 5 μg/kg. The use of toxic solvents such as benzene and chloroform is minimized. Flammability can be reduced by the substitution of Freon 113 for hexane.

DETERMINATION OF AFLATOXIN LEVELS IN CORN

Methods of determining aflatoxin levels in corn involve three steps: extraction, partial purification of extract, and measurement of quantitation of the mycotoxin, Table 9. An ideal extraction solvent would remove all of the aflatoxin and no substance that would interfere with the quantitation step. Extracted substances that interfere with the quantitation step are removed by a puri-

TABLE 9. METHODS OF DETERMINING AFLATOXIN LEVELS IN CORN

Extraction solvent	Purification	Method of measuring aflatoxin	Reference
Chloroform-water (10:1, v/v)	Silica gel chromatography	Thin layer chromatography (TLC)	*40*
Methanol-water (75:25, v/v)	Solvent transfer ammonium sulfate solution, hexane, methylene chloride	TLC or HPLC	*527, 530*
Methanol	Solvent transfer-ammonium sulfate solution, hexane, methylene chloride	Two-dimensional TLC	*11*
Acetone-water (85:15, v/v)	Solvent transfer, acidic alumina, silica gel chromatography	TLC	*49*
Methanol-water (80:20, v/v)	Treatment zinc acetate solution; basic alumina chromatography	High pressure liquid chromatography (HPLC)	*341*
Chloroform-water (10:1, v/v)	Silica gel chromatography	Reverse phase HPLC	*59*
Chloroform-water (10:1, v/v)	Silica gel chromatography	HPLC	*389*
Methanol-10% sodium chloride (4:1, v/v)	Zinc acetate solution, small silica gel column	HPLC	*477*
Methanol-water (80:20, v/v)	Ammonium sulfate solution, Sep Pak silica cartridges or small HPLC grade silica gel column	HPLC	*589*
Methylene chloride-water (10:1, v/v)	Small silica gel column	TLC	*348*
Acetonitrile-water (20:3, v/v)	Sep-Pak silica cartridge	Reverse phase HPLC	*137*
Methylene chloride-formic acid	Sep-Pak C-18 cartridge	Fluorometric-iodine method	*156*
Methylene chloride-water (5:1, v/v)	Basic cupric carbonate	Reverse phase HPLC	*162*

fication or a clean-up step. Treatments with inorganic salts, solvent transfers, or column chromatography have been used to clean up extracts. The first reported method utilized TLC to determine amounts of aflatoxin in extracts. More recently, high-pressure liquid chromatography (HPLC) has been used.

It was suggested that dockage or broken corn-foreign material (BCFM) in corn contains most of the aflatoxin and that an analysis of BCFM would be representative of the contamination of an entire lot *(310)*. However, more complete studies of naturally contaminated corn revealed that BCFM accounted for less than 10 percent of the aflatoxin content *(540, 545, 546)*. A study of the segregation of aflatoxin-contaminated corn from a lot using differences in buoyancy showed that contaminated corn was less dense than uncontaminated corn *(294)*. Analysis of the buoyant fraction would increase the sensitivity of aflatoxin detection, but the analysis would not be representative of the lot.

The CB method originally developed for peanuts was approved in Official First Action for corn by the AOAC *(40)* and the AACC *(17)* after an international collaborative study *(557)*. In the 1979-1980 season, the coefficient of variation in total aflatoxin of corn samples sent out by the Smalley test sample series and analyzed by the CB method was 39.6 percent between laboratories and 17.7 percent within laboratories *(405)*. Of 35 participants, 23 used the CB procedure. The International Check Sample Program provided a corn meal sample free of charge for aflatoxin analysis to 182 laboratories requesting samples *(211)*. Of the 139 laboratories from 34 countries who provided results, 47 used the CB method; the coefficient of variation of results obtained by all the methods used was 73 percent.

The disadvantage of the CB method is that large quantities of chloroform, an expensive and a relatively toxic solvent, and ether, a flammable solvent, are used. To overcome the disadvantages, Lee and Catalano *(348)* substituted methylene chloride for chloroform and cleaned up a smaller aliquot of the original extract on a small chromatography tube (Glass Econo-Column, 10 mm id, 300 mm long, Bio-Rad Laboratories), thereby saving solvents.

In an effort to avoid "large quantities of expensive, flammable, and/or toxic solvents," Seitz and Mohr *(530)* developed a method for assaying aflatoxin in corn in which the toxin was extracted with methanol-water (75:25, v/v). The initial extract was partially purified by treatment with ammonium sulfate solution and solvent distribution. Quantitation was accomplished by TLC. Alexander and Baur *(11)* used the Seitz-Mohr method on corn and dry-milled corn products, but they had to quantitate by two-dimensional TLC when assaying extremely moldy samples. Barabolak *(49)* reported a procedure for determining aflatoxin in corn and wet-milled corn products that used aqueous acetone as an extractant and an ammonium sulfate purification, but he had to add another clean-up step before TLC on silica gel.

So far, the methods described have involved measurements of aflatoxin by TLC. The zones containing the fluorescent toxins are measured by visual comparison of the unknowns with known amounts of aflatoxins applied to the TLC plates. Very sophisticated automatic densitometers are available to scan plates and recorders with integrators, or a computer system can give values of fluorescence in a zone. The reality is that companies that have to monitor products around the clock cannot afford people on every shift with the ability to maintain complicated equipment. So quantitation by visual comparisons with standards is still a necessity. Actually the results are just as accurate. Dickens et al. *(163)* reported a "Spotmeter" to measure aflatoxin on TLC plates. It was stated that the "Spotmeter" made measurements in one-fourth the time required for commercial densitometers and would cost one-sixth as much. However, the measurements reported were those of standards. The percentage errors in measurement reported for the commercial densitometer were far higher than one would expect.

A comparison was made by Shotwell and Goulden *(543)* of the three methods used for the analysis of aflatoxin in corn—the official first action CB method, the Pons method for cottonseed, and the BF method developed for peanuts. Both spiked and naturally contaminated corn samples were analyzed. Recoveries were much lower with the BF method than with the CB method. Recoveries of aflatoxin B_1 with the Pons method were satisfactory in samples containing less than 50 μg/kg, but recoveries were only 50 percent for samples containing 50 and 100 μg/kg; however, no aflatoxin G_1 was detected by the Pons method in a naturally contaminated sample that did have G_1. Reducing the amounts of solvents used in the official AOAC method was also studied. The extraction solvent, chloroform, could be reduced from 250 ml to 150 ml and washing and eluting solvents for column chromatography could be reduced by half without affecting this study.

Since HPLC equipment has become available, a number of laboratories have quantitated aflatoxins in cleaned-up extracts of corn by HPLC, Table 9. The advantages are that quantitation can be automated and degradation of aflatoxins that sometimes takes place on TLC plates is avoided. The number of participants reporting results in collaborative studies by HPLC methods is small *(211, 405)*. However, their results compare closely with those using TLC methods. At least eight HPLC methods have been reported for corn from 1975 through 1981. Extracting solvents have been methanol-water combinations, chloroform or methylene chloride plus water, or acetonitrile-water. Extracts have been cleaned up by ammonium sulfate, zinc acetate, or basic cupric carbonate treatment, and by silica gel, and/or alumina column chromatography and Sep-pak silica gel cartridges (Waters Associates, Milford, MA 01757), or Pasteur pipet columns packed with 37-75 μm silica gel Porisil (Waters Associates).

A number of liquid chromatographic systems are available with different pumps, injectors, and detectors. The detectors and conditions used to measure aflatoxins are summarized in Table 10. For more details such as flow rates, references are given to individual papers. Aflatoxins are detected in column eluants by ultraviolet absorption or fluorescence. Detection limits can be lowered by preparing the highly fluorescent water adducts of aflatoxins B_1 and G_1 by treatment with trifluoroacetic acid. Normal phase columns are packed with microparticulate silica gel. Reverse phase HPLC uses columns packed with octadecylchlorosilane bonded to silica gel, known as C_{18} columns.

In 1979, Davis and Diener *(151)* reported a fluorometric-iodine (FL-I) method for measuring aflatoxin in corn. The extraction solvent was methylene chloride with 1% methanol by volume. The solvent was evaporated and aflatoxin was transferred to water. After defatting with hexane and clarifying on a polyethyleneimine-cellulose column, the aqueous solution was treated with iodine to form the highly fluorescent iodine derivative of aflatoxin. Fluorescence was measured with the photofluorometer and filters described in the FL-IRS method. The sensitivity, precision, and accuracy of the FL-I method compares to those of the official AOAC methods, but it has not been studied collaboratively, Table 11. In 1981, Davis et al. improved the method by packing ground corn samples acidified with 5% formic acid in butt tubes for extraction *(156)*. Samples were defatted under suction with hexane or petroleum ether. Aflatoxins were extracted with methylene chloride. Extracts can be checked for aflatoxin by TLC on silica gel plates or slides. Before the iodine treatment, extracts were purified on Sep-pak C_{18} cartridges.

A detection limit of aflatoxin in corn of 0.1 μg/kg has been reported using a laser fluorometric technique *(166)*. The corn extract was prepared by the method of Seitz and Mohr *(530)*, which includes extraction with water-methanol and purification with an ammonium sulfate treatment and hexane. To attain the low detection limit, more impurities were removed by preparative TLC before forming the water adduct for reverse phase HPLC. The sensitivity

TABLE 10. CONDITIONS FOR HIGH PRESSURE LIQUID CHROMATOGRAPHY

Detection	Column packing	Solvent	Reference
Fluorescence; ultraviolet (365 nm)	μ Porisil silica[a]	Chloroform-methylene chloride (75:25 v/v) containing 0.5% methanol by volume	*527*
Ultraviolet (350 nm)	Corasil II, 37-50 μm[a]	Chloroform-heptane-methanol (54.5:45:0.5 v/v)	*341*
Ultraviolet (350 nm)	Bondapak C-18/Porasil (37-50 μm)[a]	Acetonitrile-water	*582*
Fluorescence[b]	10 μm Spherisorb ODS, C18[c]	Water-acetonitrile-methanol (15:3:2, v/v)	*59*
Fluorescence with packed cell ultraviolet (365 nm)	Zorbax Sil silica[d]; silica AB-5, 5.5 μm[e]	Toluene-ethyl acetate-formic acid-methanol (89.0:7.5:2.0:1.5, v/v)	*389*
Fluorescence with packed cell	Porisil silica gel (10 μm)[a]	Water-saturated chloroform-cyclohexane-acetonitrile (25:7.5:1, v/v) with added 1.5% absolute ethanol or 2.0% 2-propanol	*477*
Fluorescence with packed cell	Zorbax Sil (5 μm)[d]	50 pct. water-saturated chloroform-cyclohexane-acetonitrile-ethanol (735:235:30:15, v/v)	*589*
Fluorescence[b]	Radial Pak 10 μm C-18[a]	Acetonitrile-water	*137*
Fluorescence[b]	Radial compression column with C-18[a]	Acetonitrile-water-glacial acetic acid (180:820:10, v/v)	*162*

[a]Waters Associates, Milford, MA 01757.
[b]Aflatoxins B_{2a} and G_{2a} were prepared from Aflatoxins B_1 and G_1 for HPLC.
[c]Spectra Physics No. 0414-4150, San Jose, CA 95134.
[d]DuPont, Wilmington, DE 19898.
[e]FD Institute, Japan.

TABLE 11. ANALYSIS OF NATURALLY CONTAMINATED CORN BY THREE METHODS

Sample No.	Aflatoxin B_1 (μg/kg)		
	CB method[a]	Pons method[a]	FL-1
1.	trace[b]	n.d.[c]	n.d.
2.	trace	n.d.	n.d.
3.	28	16	23
4.	22	16	22
5.	28	22	30
6.	30	20	33

[a]Official AOAC methods.
[b]Data represents the average of two replications.
[c]None detected.

of the method is based on the detection of laser-induced fluorescence in a droplet (4 μl) of eluant from the HPLC column. The feedback-stabilized He-Cd laser (Liconix Inc., Mountain View, CA 94043), Model 405 UV, provides an 8-mW amplitude modulated 325-nm beam for excitation of aflatoxin fluorescence. Although the laser fluorometer is commercially available, the flowing droplet detection cell is not. The author expects that laser fluorometry in conjunction with HPLC will prove useful in trace detection problems.

Aflatoxin in corn can be determined by an enzyme-linked immunosorbentassay (ELISA) and a solid-phase radioimmunoassay (RIA) *(197)*. Samples were spiked at 2.9-43.2 μg/kg aflatoxin B_1 and extracted by the CB method using chloroform-water. Extracts were concentrated to dryness and the residues were taken up in methanol and diluted with phosphate-buffered saline with Tween 20 before analysis by either ELISA or RIA. At 5.8 μg/kg or greater, recoveries for aflatoxin B_1 in corn samples were 80.0 percent by ELISA and 61 percent by RIA. Overall results indicated that ELISA gave more consistent data, relatively lower standard deviations, and lower coefficients of variation than did RIA, Table 12. The ELISA is considerably faster, safer, and less expensive than RIA. The microtiter plate used in ELISA is the basis of a number of diagnostic kits. Analysis of a naturally contaminated yellow corn sample revealed that the result obtained by ELISA was comparable to that obtained by established chemical methods.

A method was developed to determine aflatoxin in roasted corn that used a Florisil column for the purification step to facilitate studies on the possibility of detoxifying contaminated corn by roasting *(534)*. Häggblom and Casper *(265)* described a method for determining aflatoxin B_1 in corn silage. They applied it to 275 samples of moldy and nonmoldy corn silage in 1976 and 1977 and found no aflatoxin. The CB method adopted by the AOAC and AACC was modified to determine aflatoxins in airborne dusts generated from naturally contaminated corn *(542)*. The method has been applied to airborne dust samples collected on farms at corn harvest and at elevators as corn was loaded and unloaded. Results will be used in an attempt to assess the potential hazard to agricultural workers handling contaminated corn.

TABLE 12. RECOVERY OF AFLATOXIN B_1 FROM CORN BY ELISA AND SOLID PHASE RIA[a]

Added ppb	ELISA		RIA	
	Rec. (ppb) ± SD	Rec. (pct.) ± SD	Rec. (ppb) ± SD	Rec. (pct.) ± SD
0[b]	0		0	
2.9	2.0 ± 1.1	69 ± 31	2.3 ± 1.4	79 ± 48
5.8	4.2 ± 1.8	73 ± 31	3.7 ± 2.4	64 ± 41
14.4	11.1 ± 1.2	77 ± 8	8.5 ± 3.7	59 ± 26
28.8	24.5 ± 5.6	85 ± 19	16.6 ± 2.2	58 ± 8
43.2	36.6 ± 10.1	85 ± 24	27.4 ± 11.8	63 ± 27
Av. rec. pct.	80.0		61.0	
Std. dev.	20.5		25.5	
Coeff. of var. pct.	25.6		41.8	
No. of replicate assays 24	16			

[a]Data at 2.9 ppb level were excluded from statistical analysis.
[b]Corn extract used for zero determination.

CONFIRMATORY TESTS FOR AFLATOXIN

For acceptance of analytical results for aflatoxin in corn, especially for regulatory purposes, proof of identity of the toxin being measured is sometimes necessary. Numerous tests have been devised for confirmation of aflatoxins and are reviewed by Nesheim and Brumley *(443)*. Included are tests based on toxicological effects observed in the duckling, zebrafish, chick embryo, *Bacillus megaterium*, and many other species. One of the confirmatory tests most widely used is the formation of the water adducts, B_{2a} and G_{2a}, of aflatoxin B_1 and G_1 by treatment with trifluoroacetic acid *(484)*. The Food and Drug Administration (FDA) stated that the chemical test, in which the water adduct is formed could be used to confirm the identity of aflatoxin *(28)*. It has been approved by the AOAC *(40)* and the AACC *(19, 20)*. Other derivatives used in confirmatory tests are acetates, condensation products, oximes, phenylhydrazones, and reduction products. In 1980, Davis and Diener reported a method to establish identity of aflatoxin by reverse phase HPLC of the highly fluorescent iodine derivative *(153)*.

Mass spectrometry (MS) is one of the most specific methods of confirmation available, but it is difficult to use at the low concentrations at which aflatoxins must be detected. At the FDA's Bureau of Foods, a MS technique based on negative ion chemical ionization was developed for confirming the identity of aflatoxin *(443)*. Tandem mass spectrometry or MS-MS promises to be a sensitive method for confirming aflatoxins in relatively crude extracts *(406)*. Mass spectrometers are coupled in a series. The targeted compound in picogram quantities in a mixture is ionized, and its characteristic ions are separated from most others of the mixture in the first MS. Selected primary ions are then decomposed by collision and, from the resulting products separated in the second MS, the compound is identified. The MS-MS technique can be used for fairly rapid screening of crude extracts of corn for a number of mycotoxins, including aflatoxin, as well as confirming the identity of aflatoxin. The technique can also be used for quantitation.

MULTITOXIN SCREENING PROCEDURES

Toxic effects in farm animals consuming moldy corn may be the result of synergism between aflatoxin and one or more other mycotoxins. Therefore, it is important to have multitoxin screening procedures available for a number of mycotoxins in corn. Screening methods have been reported for aflatoxin and one or more of the following mycotoxins: citrinin, diacetoxyscirpenol, fusarenon X, luteoskyrin, neosolaniol, patulin, penicillic acid, penitrem A, ochratoxins, rugulosin, sterigmatocystin, T-2 toxin, and zearalenone, Table 13. All of the methods use TLC to detect mycotoxins in partially purified extracts.

CONCLUSION

A number of effective, reliable methods of detection and determination of aflatoxin in corn are available. The BGYF presumptive test is widely used by farmers, elevator owners, and industry to identify suspect corn lots that might contain aflatoxin. For laboratories with a minimum amount of equipment and a need for results in a minimum amount of time, the Holaday-Velasco minicolumn method for aflatoxin has been adopted for aflatoxin in corn by the AOAC and AACC. There is a need for a method of determining aflatoxin levels that is less expensive than the approved CB method and that uses less toxic solvents. There are several promising quantitative methods that should be evaluated in collaborative studies for AOAC and AACC approval. One method—the ELISA—should be investigated further to develop kits that can be used by almost any technician. Research is needed on tandem MS to determine the extent of its applications and limitations.

TABLE 13. MULTITOXIN SCREENING METHODS FOR CORN[a]

Mycotoxins other than aflatoxin	Extraction solvent	Purification of extracts	Reference
Zearalenone, ochratoxin A	Chloroform-water (10:1, v/v)	Silica gel chromatography	*199*
Ochratoxin, sterigmatocystin	Chloroform-methanol-hexane (8:2:1, v/v)	Silica gel chromatography	*623*
Zearalenone, ochratoxins, Sterigmatocystin, patulin	Acetonitrile-potassium chloride (4 g/100 ml water) (9:1, v/v)	Solvent transfer	*579*
Zearalenone	Methanol-water (60:40, v/v)	Solvent transfer	*594*
Zearalenone, ochratoxins, Sterigmatocystin	Chloroform-water (10:1, v/v)	TLC double development	*263*
Zearalenone	Methanol	Ammonium sulfate solution, solvent transfer	*529*
Zearalenone, ochratoxin, penicillic acid, citrinin	0.5 N. Phosphoric acid-chloroform (1:10, v/v)	Silica gel chromatography	*666*
Zearalenone, ochratoxin, Sterigmatocystin, patulin	0.1 M Phosphoric acid-chloroform (1:10, v/v)	Sephadex gel filtration	*319*
Zearalenone, ochratoxin A	Acetonitrile-water (90:10, v/v)	Solvent transfer	*48*
Zearalenone, ochratoxin A, sterigmatocystin, citrinin T-2 toxin, diacetoxyscirpenol, neosolaniol, fusarenon X, luteoskyrin, rugulosin	20% Sulfuric acid-4% potassium chloride-acetonitrile (2:20:178, v/v)	Silica gel chromatography	*585*
Zearalenone, ochratoxins, sterigmatocystin, patulin, citrinin, penicillic acid, T-2 Toxin, diacetoxyscirpenol, penitrem A	Acetonitrile-4% potassium chloride (9:1, v/v)	Solvent transfer	*227*
Ochratoxin A, sterigmatocystin, Zearalenone, T-2 toxin	Acetonitrile-4% potassium chloride by volume (90:10, v/v)	Dialysis membrane, 2-dimensional TLC	*462*

[a]The method of detection is thin layer chromatography (TLC).

In "Aflatoxin and Aspergillus flavus in Corn," eds. Urban L. Diener, Richard L. Asquith, and J. W. Dickens, Proc. Symp. held in Atlanta, Ga., Jan. 26-27, 1982. South. Coop. Ser. Bull. 279: 38-45. February 1983. (Published at Alabama Agricultural Experiment Station, Auburn University, Alabama)